

Letter to the Editor

Freezing does not alter antigenic properties of fresh fruits for skin testing in patients with birch tree pollen-induced oral allergy syndrome

To the Editor:

The oral allergy syndrome (OAS) describes an allergic reaction that occurs on ingestion of certain fresh fruits and vegetables in pollen-sensitized subjects. The culprit allergens found in associated fresh foods are rapidly inactivated by digestion and cooking. As a result, symptoms are usually limited to the mouth and throat and preferably observed with raw forms of the food. When evaluating patients with OAS, skin prick tests (SPTs) with commercial extracts will often yield negative responses because the allergens are thought to be altered in the fabrication process of food extracts.^{1,2} Specific serum IgE (ssIgE) assays are also of limited value,³ and although recombinant allergens appear to be promising, they are not yet widely available.⁴

At present, the preferred method for evaluating patients with OAS is the use of SPTs with fresh fruits and vegetables using the prick-prick technique, which consists of pricking the fruit directly with a lancet and then using the same lancet immediately for the skin prick.⁵ This method has been shown to be much more sensitive than SPTs with commercial extracts for most foods implicated in the syndrome.⁶

A practical limitation to this approach is the access to fresh food at the clinic. One solution is to have the patient return for a second appointment with his own fresh food, but this is time consuming for both the patient and the physician and delays diagnosis. Another potential pitfall is that the culprit fruit might not be available in its fresh form depending on the harvest season.

Although the effect of cooking is well established, much less is known about the effect of freezing on the allergenic properties of the proteins responsible for OAS. During freezing, soluble proteins are progressively entrapped in newly formed crystals and might experience shear tension at the ice-liquid interface.⁷ Although this phenomenon has been shown to decrease the enzyme activity of some soluble proteins, the effect on antigenicity has not been studied. We hypothesized that frozen fruits would have the same value as fresh fruits for the purpose of skin testing patients with OAS.

Twenty-three patients with OAS were recruited from the allergy clinic at our center. Inclusion criteria were as follows: (1) sensitivity to birch pollen on skin testing, (2) oral symptoms to a plant food related to birch, (3) and sensitivity to this fresh plant food on skin testing. Demographic data and medical history were collected for each subject. They were also asked to fill out a questionnaire regarding their upper respiratory tract symptoms, and scores for rhinoconjunctivitis symptoms were calculated.⁸

Fruits were bought fresh and frozen in a regular home freezer at -18°C for 48 to 72 hours. Frozen fruits were thawed at room temperature for 15 minutes before evaluation. Skin tests were performed with aeroallergens and commercial fruit extracts (Omega Laboratories, Montreal, Quebec, Canada), as well as with fresh and thawed fruits, by using the prick-prick method.⁵ ssIgE levels were measured for each fruit by using UNICAP technology (Phadia, Uppsala, Sweden). Twenty-three control subjects without symptoms of OAS and with negative SPT responses to

TABLE I. Clinical characteristics of subjects

	Group 1 (n = 23)	Group 2 (n = 19)	Control subjects (n = 23)
Male sex	15 (65%)	5 (26%)	10 (53%)
Age (y)	32.9 \pm 9.1	31.4 \pm 12.1	32.4 \pm 13.1
Rhinitis	23 (100%)	19 (100%)	5 (26%)
Asthma	13 (57%)	9 (47%)	1 (4%)
Eczema	12 (52%)	5 (26%)	5 (22%)
Symptoms of OAS			
Oral	23 (100%)	19 (100%)	0
+ Gastrointestinal	5 (22%)	4 (21%)	0
+ Systemic	4 (17%)	7 (37%)	0
+ Life-threatening	2 (9%)	5 (26%)	0
Rhinoconjunctivitis score	2.5 \pm 1.0	2.3 \pm 1.4	NA
Birch skin test (mm)	10.6 \pm 4.9	10.8 \pm 3.5	0
Avoidance of fresh fruits	20 (87%)	17 (89%)	0

Group 1 and control subjects were tested with fruits frozen for a short period (48-72 hours).

Group 2 subjects were tested with fruits that underwent prolonged freezing (>6 months).

NA, Not applicable.

birch pollen were also recruited from the clinic staff and underwent skin testing. Agreement between allergy tests was analyzed by using the Pearson correlation coefficient (PCC). Sensitivity was calculated as described elsewhere.⁹ All statistical analyses were performed with the SPSS software package (SPSS, Inc, Chicago, Ill). The study was reviewed and approved by the local ethics committee. All subjects provided written informed consent before enrollment into the study.

Demographic and clinical data are presented in Table I. Table II shows allergy test results and agreement between tests for each fruit. Although correlation between fresh and frozen fruit SPT responses is shown to be systematically high and significant, correlation between fresh fruit and extract SPT responses remains very low and seldom significant. Somewhat lower PCCs were found between fresh and frozen plum, peach, nectarine, and Golden Delicious apples when compared with other fruits. This could be explained in part by the fact that these fruits elicited some of the largest SPT responses because marginally high results are expected to have lower reproducibility (regression toward the mean phenomenon).

When considering all Bet v 1-related fruits (ie, excluding cantaloupe and watermelon, which are associated with allergy to the birch allergen Bet v 2), the size of the wheal from fresh fruit tests did not correlate with that obtained with birch tree extracts or with rhinoconjunctivitis scores (PCC, 0.011 and -0.021 , respectively; not significant). There was, however, a poor but significant correlation between fresh fruit SPT responses and ssIgE values (PCC, 0.227; $P = .003$). Similar findings were observed for watermelon and cantaloupe, although correlation with ssIgE levels was nonsignificant, possibly because of the small numbers of patients sensitized to these fruits.

Overall sensitivities of SPTs with fresh (81%) and frozen (82%) fruits were similar and in line with those reported in the literature.⁶ Overall sensitivity of SPTs with fruit extracts (12%) and ssIgE levels (69%) also fell within the expected range. Interestingly, ssIgE levels and extract SPT responses did not correlate

TABLE II. Allergy test results of subjects with OAS

Fruit	Symptoms	Extract†		Raw†		Frozen†		PCC (<i>P</i> value)	
		+	Mean (mm)	+	Mean (mm)	+	Mean (mm)	Raw vs frozen	Raw vs extract
Group 1: Short-term freezing (n = 23)									
McIntosh	22	4	0.86 ± 1.5	14	3.43 ± 2.6	12	2.61 ± 2.0	0.774 (<.0005)	0.081 (.720)
Spartan	22			15	3.09 ± 2.5	15	2.74 ± 2.0	0.764 (<.0005)	0.218 (.329)
GD	17			20	4.34 ± 2.4	18	3.56 ± 2.0	0.571 (.001)	0.243 (.181)
GS	19			18	4.00 ± 2.0	20	4.17 ± 2.0	0.829 (<.0005)	0.077 (.732)
Pear	10	3	0.50 ± 1.1	16	3.30 ± 2.2	18	3.43 ± 2.1	0.992 (<.0005)	−0.110 (.627)
Nectarine	13	NA	NA	20/20*	5.05 ± 2.3	18/20*	4.60 ± 2.3	0.527 (.017)	NA
Peach	6	1	0.27 ± 0.9	19/20*	5.80 ± 2.8	19/20*	4.70 ± 2.3	0.407 (.075)	−0.154 (.528)
Plum	9	3	0.73 ± 1.9	20	4.35 ± 1.9	20	3.87 ± 2.4	0.505 (.014)	0.162 (.471)
Avocado	2	1	0.45 ± 1.0	8	1.43 ± 2.0	7	1.61 ± 2.5	0.902 (<.0005)	−0.136 (.546)
Kiwi	6	1	0.32 ± 1.1	12	2.87 ± 2.5	12	2.30 ± 2.1	0.903 (<.0005)	0.154 (.494)
WM	3	3	0.55 ± 1.4	5	1.17 ± 1.9	5	1.00 ± 1.6	0.908 (<.0005)	0.410 (.058)
Cantaloupe	3	3	1.04 ± 2.3	4	0.87 ± 1.5	5	1.13 ± 2.0	0.946 (<.0005)	0.419 (.052)
Cherry	12	3	0.36 ± 0.9	20/20*	5.10 ± 2.7	20/20*	4.36 ± 2.3	0.846 (<.0005)	0.128 (.603)
Apricot	6	3	0.73 ± 1.8	19/20*	3.65 ± 1.9	18/20*	3.20 ± 1.7	0.793 (<.0005)	0.380 (.109)
Group 2: Prolonged freezing (n = 19)									
McIntosh	16	2	0.43 ± 1.23	6	2.67 ± 2.1	6	2.46 ± 1.9	0.915 (<.0005)	0.224 (.371)
Cortland	16			13	3.87 ± 2.0	14	3.69 ± 1.7	0.895 (<.0005)	0.421 (.085)
GD	15			15	4.41 ± 2.0	13	4.61 ± 2.2	0.844 (<.0005)	0.380 (.109)
GS	12			14	4.25 ± 2.4	14	4.37 ± 2.1	0.808 (<.0005)	0.219 (.369)
Pear	10	0	0	12	3.19 ± 2.1	14	3.81 ± 1.5	0.752 (<.0005)	NC
Red plum	12	0	0.05 ± 0.23	14	4.07 ± 1.6	13	3.53 ± 1.6	0.806 (<.0005)	0.089 (.716)
Black plum	12			12	3.54 ± 2.1	12	3.52 ± 1.3	0.855 (<.0005)	0.127 (.604)
Peach	17	2	0.58 ± 1.08	16	4.61 ± 1.5	18	5.06 ± 1.7	0.862 (<.0005)	0.170 (.488)
Cherry	19	0	0	14	4.54 ± 1.7	18	5.01 ± 2.0	0.738 (<.0005)	NC

GD, Golden Delicious (yellow apple); GS, Granny Smith; NA, not available; NC, not calculable; WM, watermelon.

*Nectarine, peach, cherry, and apricot could not be tested in 3 subjects from the first group because of seasonal availability.

†A positive test response was defined as a diameter of 3 mm or greater.

with the severity or systemic nature of symptoms, as would have been expected, possibly because of the small size of the sample.¹⁰

Control subjects all had negative responses to both fresh and frozen extracts, therefore excluding an irritating effect of fresh or frozen fruits. Although many patients reported a stinging sensation to both fresh and frozen kiwifruit, this did not translate into positive papules.

The experiment was repeated with a second group of patients with OAS (Table I) by using a panel of fruits that had been frozen for at least 6 months to evaluate whether long-term freezing had a different effect on antigenicity (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org). The oldest fruit used was a 2-year-old Golden Delicious apple (see Fig E3 in this article's Online Repository at www.jacionline.org). All SPTs were repeated 3 times and the mean was used for analysis to compensate for the internal variability of skin tests and regression toward the mean. As shown in Table II, results replicated well. When using means of 3 tests, correlation coefficients for frozen plum, peach, and yellow apple were similar to other fruits.

Although short-term freezing was associated with slightly lower test values than raw fruits (mean difference, −0.36 mm; $P < .0005$), it was surprisingly not the case with prolonged freezing (mean difference, +0.14 mm; not significant). The higher values observed with long-term frozen peach and cherries (Table II) could result from the comparison of fruits from different seasons and imported from different regions and hence with different antigenicities.

From these results, we conclude that freezing and thawing a fruit does not alter its antigenic properties for the purpose of

skin testing patients with OAS. SPTs with frozen fruits could be a reliable alternative for the diagnosis of OAS when fresh fruits are not available. Frozen fruits can be kept in a regular home freezer at the clinic, and their use could prevent patients from needing a subsequent appointment for diagnosis to be confirmed.

We thank Ms Méline Jean for photography.

Philippe Bégin, MD, MSc^a

Anne Des Roches, MD, FRCPC, FAAAAI^b

Mélanie Nguyen, MD, FRCPC^a

Marie-Soleil Masse, MD, FRCPC^a

Jean Paradis, MD, FRCPC^a

Louis Paradis, MD, FRCPC, FAAAAI^{a,b}

From ^aCentre Hospitalier de l'Université de Montréal, Department of Medicine, and

^bCentre Hospitalier Universitaire Sainte-Justine, Department of Pediatrics, Service of Allergy and Clinical Immunology, Montreal, Canada. E-mail: lparadis@aei.ca.

Disclosure of potential conflict of interest: M.-S. Masse receives research support from Merck Frost, Laboratoires Omega, AstraZeneca, and Novartis. The rest of the authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2011.01.028



FIG E1. Fruits frozen for 6 months.



FIG E2. Fresh fruits.



FIG E3. Golden Delicious apples fresh, after 6 month of freezing, and after 2 years of freezing and extensive pricking.